35. Epidemiology and control of *Salmonella* in the pork production chain: the approach in a high prevalence country (Spain).

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Abstract

The present study summarises the insights yielded by different studies focused on the epidemiology of the infection by *Salmonella* through the pig production (breeders, finishers and post-farm stages). The final objective is to determine the best strategies for successful control of *Salmonella* in a highly prevalent country such as Spain. The study in breeders evidenced a high seroprevalence. There were differences among some of the 10 most common serotypes detected in breeders compared to previous studies in finishers, although the two most common were shared by both populations. Type of feed and type of floor were the factors associated to the presence of *Salmonella*. A *Salmonella* Typhimurium inactivated vaccine and two organic acids demonstrated to be good tools to reduce the prevalence in finishers. Finally a study of the contamination in post-farm stages evidenced the high contamination of transport, lairage and the contamination of the slaughter line, accounting for 70% of carcass cross-contamination.

Introduction

Salmonella is one of the most important food-borne pathogens and pork one of the main sources of infection for humans (EFSA, 2014). For this reason control programmes in swine production are common in Europe. Spain is one of the main producers of pork in Europe and previous studies have shown that *Salmonella* prevalence in finishing pigs is relevant (García-Feliz *et al.*, 2007). Before considering establishing any control programme, more research is needed to establish the best approach to control efficiently the pathogen through the pig production chain. The present study summarises the most relevant findings in recent years on the study of the epidemiology of *Salmonella* in Spanish pigs and pork.

Material and Methods

Studies design

- Breeders: Cross-sectional study to determine the bacteriological and serological prevalence as well as the risk factors associated to the infection in sows by the association of the results obtained and the answers to a questionnaire formulated on each farm.
- Finishers: Considering the high prevalence determined by previous studies, we evaluated the efficacy of two control strategies on highly infected farms. First, the administration of organic acids (table 1), (a mixture of lactic-propionic-formic-acetic 0.035% in drinking water and potassium-diformate 0.5% in feed; and second the usefulness inactivated-vaccine (*S.*Typhimurium).
- Post-farm: Study of the epidemiology of the infection in transport, lairage, and slaughtering. Double study (1) monitoring of 16 batches from farm to slaughterhouse (2) study of the environmental contamination in 4 abattoirs (lairage, slaughter line, carcasses and cutting plants).

Methods

Salmonella isolation: Following the annex D of the ISO method 6579/2007 for the isolation of *Salmonella* from primary production samples.

- Blood analysis: Using a commercial ELISA (Herdchek Idexx). Results were analysed at 20%, 40% OD cut-offs.
- Typing methods: *Salmonella* isolates were serotyped by slide agglutination following the Kauffmann-White scheme. Further typing was performed by PFGE (*S.* Derby) and MLVA (*S.* Typhimurium). More information in (Arguello *et al.,* 2013a)

Results

Breeders: The study performed in 309 herds evidenced, by serology and bacteriology, the presence of *Salmonella* in 60% of the herds. *S.* Rissen, *S*. Typhimurium and *S.* Derby were the main serotypes detected. From the 10 most common serotypes found, four were different to a previous study in finishing pigs (Garcia-Feliz *et al.*, 2007). The analysis of risk factors revealed that pelleted feed and individual housing were linked to herds with *Salmonella*, while slatted floor was pointed out as protective factor.

Finishers: Three trials were performed using two different combinations of organic acids in water or feed (trials A, B and C). The use of organic-acids during the last 6 weeks of finishing reduced the risk of finding seropositive pigs and faecal shedding at the end of the treatment, regardless of the acid used or the administration via (water/feed). The protection conferred by a *S*. Typhimurium-inactivated vaccine was demonstrated by the reduction of shedders (six times compared to control animals), when a homologous infection was established; while no protection was achieved in herds infected by serotypes different from *S.* Typhimurium (*S.* Rissen infection).

Post-farm

The monitoring study evidenced an increase in contamination from farm (faeces) to the slaughterhouse (caecum content, lymph nodes and carcasses) in the tracked animals (table 2). Carcasses prevalence was 7 times higher than shedders prevalence on farm. The increase in the number of positive pigs in caecum and lymph nodes compared to faecal samples was linked to new infections occurring in holding pens. Molecular typing of isolates obtained from the carcasses revealed that cross contamination occurred in 75% of the positive carcasses.

The evaluation of the environment in four slaughterhouses confirmed the contamination of the lairage through the day (table 3). Contamination in the slaughter line was detected in manual activities such as evisceration or lard and fat removal. Carcass prevalence was reduced after chilling and cooling. Molecular typing of the isolates revealed that there was a continuous flow of *Salmonella* introduced by positive pigs, thus molecular profiles (PFGE or MLVA) detected in the slaughter line activities or carcasses at the beginning of the day were different to half working or the end of the working day.

Discussion

As a result of the control of *Salmonella* in poultry and laying hens, the incidence of human cases has dropped down significantly (EFSA, 2014). In contrast, the role of pork products has been enhanced due to the lack of compulsory control programmes in pig production. The epidemiology of *Salmonella* is complex. Before establishing any control programme, any country should perform studies to evaluate which stages of the production chain should be included in the control. In the present study, we summarise the main results from a number of studies performed throughout the pig production chain in Spain (Arguello *et al.*, 2012; 2013a; 2013b; 2013c).

The cross-sectional study evidenced a high prevalence in breeders. There is more research needed to establish the role of breeding pigs in the transmission of the infection and requirements of control in this stage. Low cost solutions such as vaccination, the use of meal instead pelleted feed or slatted –floors instead of concrete could reduce the *Salmonella* prevalence in breeding herds.

The high prevalence in finishing pigs determined in previous studies (Garcia-Feliz *et al.*, 2007) makes of paramount importance to find strategies to reduce the burden of *Salmonella* on this stage. Organic acids have been pointed out as a potential tool to control the pathogen (Creus *et al.*, 2007). Two organic acids were tested and results support previous studies. The results of the study show that strategic administration of effective organic acids can reduce the prevalence of *Salmonella* before slaughtering. A similar result was obtained by an inactivated *S.* Typhimurium vaccine on farms infected by this serotype. Vaccines are another useful tool to control the infection (Arguello *et al.*, 2013b; De Ridder *et al.*, 2014).

The post-farm studies evidenced and highlighted their importance in a potential control programme in Spain. Special attention should be paid to the cleaning and disinfection of transport and lairage to minimise new infections in both stages. At the same time the studies stressed the importance of including these stages in a potential control programme, by the inclusion of improved cleaning protocols and establishment of CCP and GMP within HACCPs.

Conclusion

In conclusion, the present manuscript summarises the information of different studies performed in Spain, the gained information together with the obtained in previous and subsequent studies should be considered to elaborate a potential control programme.

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Table 1. Characteristics of the treatments on farms participating in three trials (A, B and C) to evaluate the effect of the administration of organic acids, by water or feed, on the prevalence of Salmonella infection at the end of the fattening.

Clinical Trial	Treatment duration[1]	Organic acid	Administration
A	40 days	Lactic (56%) Formic (23%) Propionic (13%) Acetic (5%)	By water 0.035 ml/l
B	52 days	Potassium diformiate	By feed 0.5 kg/Tn
C	49 days	Potassium diformiate	By feed 0.5 kg/Tn

Table 2. *Salmonella* prevalence in the samples collected in the monitoring of 16 batches from farm to the abattoir.

Stage	Farm	Transport	Lairage	Caecum	MLN[1]	Carcass
Prevalence	5.6%	23.7%	85%	33%	18.2%	57.5%

[1] MLN. Mesenteric lymph nodes

Table 3. Mean prevalence of *Salmonella* obtained in the environmental study performed in four Spanish slaughterhouses.

Abattoir	Lairage	Slaughter Line	Carcass
A	80	10	35
B	76	56	36
C	66	31	38
D	75	49	47